Sponge community structure and disease prevalence on coral reefs in Bocas del Toro, Panama

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Abstract: Sponges are sessile, filter-feeding organisms that are sensitive to both biotic and abiotic components of their environment and are therefore likely to be impacted by environmental stressors. For this reason, sponges are useful as bioindicators of changing environmental conditions. The present study characterized sponge diversity, abundance and disease prevalence on three reefs in Bocas del Toro, Panama. The reefs were similar in general characteristics, however, one site was located just offshore of a village where anedoctal reports suggest that "black water" outflow (sewage, road pollution, and solid waste dumping) occurs. Overall, 51 species and 2532 individual sponges were identified. Analysis of similarity indicated significant differences in sponge community structure between the sites. The site nearest Saigon village had significantly fewer species per quadrat, although total number of individuals and number of individuals per quadrat were similar between this site and the more distant, upstream site (Punta Caracol). Evenness (J) and diversity (H') were significantly reduced at Saigon, as was the slope of the species-area curve. Dominant species also differed among sites, with the most abundant species at Saigon considered rare at the two upstream sites. Only *Niphates erecta* was among the five most dominant species at all three sites; *Aplysina fulva*, which dominated the upstream sites and is known to be sensitive to stress, was rare at Saigon, and *Chondrilla nucula*, another stress-intolerant species, was only dominant at Casa Blanca. *Hymeniacidon* sp., on the other hand, dominated the reef only at Saigon. *Aplysina* red band syndrome (ARBS) was present at all three sites, but prevalence was higher and more variable at Saigon. Differences in sponge community structure and disease prevalence at these three sites in Bocas del Toro, Panama, may be indicative of differences in environmental conditions on these reefs.

Keywords: biodiversity, disease, disturbance, sponge community structure

Introduction

Caribbean coral reefs have undergone dramatic changes over the past several decades, resulting in shifts from coral- to macroalgal-dominated communities (Porter and Meier 1992, Hughes 1994, Jackson 1997, Hughes *et al.* 2003, Aronson *et al.* 2004). The cause of these changes is presumably multi-faceted, and both natural and anthropogenic factors are clearly important (Hughes *et al.* 2003, Mumby *et al.* 2006). Corals have been the primary focus of most studies that have catalogued these shifts in community structure. Although corals are clearly susceptible to the effects of nutrient enrichment, pollution, turbidity and sedimentation (Dubinsky and Stambler 1996, Fabricius 2005, Kuntz 2005), other benthic invertebrates may exhibit differential responses to these potential stressors.

Next to corals, sponges are the most important macrofauna on Caribbean coral reefs (Wulff 2001, Diaz and Rützler 2001, Rützler 2004). Sponges are sessile, filter-feeding organisms that are sensitive to both biotic and abiotic components of their environment and are therefore likely to be impacted by environmental stressors. An increasing number of studies has documented variations in sponge community structure correlated with water quality parameters. In some cases, sponge biomass may increase with proximity to runoff (Zea 1994); however, it appears that increasing amounts of stress (e.g. increasing concentrations of organic pollutants) result in reduced sponge diversity, leaving only specialists behind (Alcolado and Herrera 1987, Muricy 1989, 1991, Muricy *et al.* 1991, Alcolado 1994, Rützler 2004, Vilanova *et al.* 2004). For this reason, sponges, both as a community and as individual organisms, have been considered useful bioindicators of changing environmental conditions.

Diseases of marine organisms, notably corals, have been reported with increasing frequency over the past few decades, particularly on Caribbean reefs (Harvell *et al.* 1999, Rosenberg and Loya 2004, Weil 2004). While the specific causes of this increase have not been fully elucidated, several studies have identified correlations between disease prevalence and local-scale processes such as pollution (Green and Bruckner 2000, Sutherland *et al.* 2004, Kaczmarsky *et al.* 2005). Although diseases of sponges are less well known, these too have been reported more frequently in recent years (Rützler 1988, Vacelet *et al.* 1994, Pronzato *et al.* 1999, Webster *et al.* 2002, Olson *et al.* 2006, Wulff 2006). The few earlier descriptions of disease among Caribbean sponges typically described outbreak conditions, such as those affecting the commercial sponge industry in the 1940s (reviewed by Lauckner 1980). Recently, Olson *et al.* (2006) described a new condition, *Aplysina* red band syndrome (ARBS), affecting approximately 10% of rope sponges in the *Aplysina cauliformis-A. fulva* complex on reefs in the Bahamas. The disease manifests as a red band composed of filamentous cyanobacteria surrounding a necrotic lesion that becomes colonized with algae. ARBS weakens the sponge skeleton, which often breaks at the site of the lesion. To date, the prevalence of ARBS in areas other than the Bahamas is unknown, and the relationship between this syndrome and environmental variables has not yet been characterized.

The Bocas del Toro Archipelago consists of a complex network of rain-forested islands, mangrove cays and peninsulas surrounding shallow bays on the Caribbean side of the isthmus of Panama (Collin *et al.* 2005). The region has complex and poorly described current patterns and is subjected to episodic severe rainfall of 3-5 m annually (Collin *et al.* 2005), resulting in high sedimentation and turbidity. Although historically home to small communities of indigenous people, there has been a long history of deforestation for agriculture (Carruthers *et al.* 2005) and, more recently, larger human settlements. Coral reefs in Bahía Almirante are well developed and highly diverse (Collin *et al.* 2005). The present study investigated sponge community structure on a coral reef offshore of a village and on two reefs upstream from this site. We examined sponge diversity, abundance and disease prevalence at all three sites, and assessed whether observed variations in these parameters were associated with variations in environmental conditions.

Materials and methods

Study sites

Three reefs were surveyed in Bahía Almirante along the south shore of Isla Colon in Bocas del Toro, Panama (Fig. 1). The three reefs were located just offshore of the village Saigon (9°20’35”N / 82°15’25”W), approximately 2 km upstream from Saigon (Casa Blanca, 9°21’32”N / 82°16’31”W) and approximately 6 km upstream from Saigon (Punta Caracol, 9°22’38”N / 82°18’10”W) (Figure 1). There are anecdotal reports of “black water” outflow (sewage, road pollution and solid waste dumping) off of Saigon (C. Schlöder, pers.obs). Within 1 km of that reef site are 200 houses, an airport and several businesses. In contrast, Casa Blanca has six houses and Punta Caracol has four houses and an eight-room hotel (Google Earth 2006). Punta Caracol is at the mouth of Big Bight, a large drainage basin, and a prevailing long-shore current flows west to east (C. Schlöder, pers. obs.), with Saigon being the easternmost of these sites. All three reefs were similar in general characteristics, consisting of a high diversity of both sponges and corals on a relatively flat slope at 5-7 m depth. Due to severe episodic pulses in rainfall, these reefs are subjected to variations in temperature, salinity, sedimentation and turbidity (Kaufmann and Thompson 2005). Visibility at all three sites during the survey dives was less than 10 m.

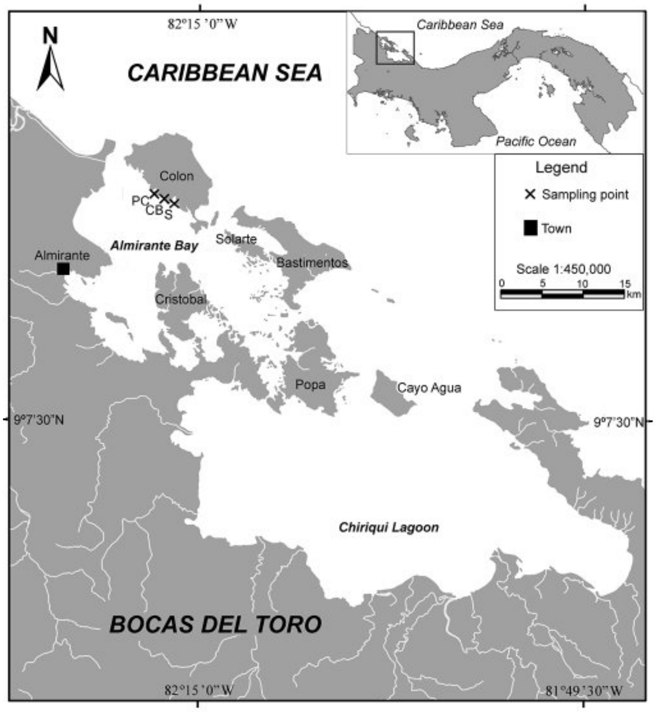

Fig. 1: Map depicting location of the three study sites at Bocas del Toro, Panama. PC = Punta Caracol, CB = Casa Blanca, S = Saigon.

Surveys

At each site, a 30 m transect line was laid along the general axis of the reef at 5 m depth. Sponge diversity and abundance were characterized from 15 1-m^2 quadrats placed on alternating sides at every other meter along the transect line. Every individual sponge within each quadrat was identified to the lowest possible taxonomic level and counted. For sponges that occurred as multiple independent ramets, even if they appeared to be part of a larger genet, each ramet was counted as a separate individual. If sponges could not be identified with certainty *in situ*, underwater photographs and/or small vouchers were collected for subsequent identification. Prevalence (percent of affected sponges) of ARBS (Olson *et al.* 2006) was assessed from three band transects (10 x 1 m) paralleling the transect line on each reef. Additional syndromes observed and the identities of their host sponges were also noted. Surveys were performed on 23-24 August 2005.

Environmental variables

In an attempt to characterize differences in water quality parameters among the sites, water samples were collected on 15 November 2005 for analyses of inorganic nutrients and on 13 July 2006 for analyses of inorganic nutrients, fecal coliform bacteria and polycyclic aromatic hydrocarbons (PAHs). There were many different parameters that could have been measured; however, these were chosen to represent potential inputs from sewage and road runoff. Although these samples were not collected concurrently with the community surveys, the relative levels of these indicators among sites is still likely to be indicative of putative risks to these coral reef communities. Nutrient and fecal coliform analyses, and preliminary processing of samples for PAHs were performed at the Smithsonian Tropical Research Institute's Bocas del Toro Research Station on Isla Colon. Concentrations of inorganic nutrients (micromolar: µM) in three replicate water samples from each site were determined in November using a chemical titration method (nitrate, nitrite, phosphate; D'Croz *et al.* 2005), and in July using a Hach DR/890 Colorimeter (nitrate, phosphate, ammonia). For the fecal coliform analyses, three replicate 1 L samples of water from each site were collected and filtered through sterile 0.45 µm nitrocellulose membrane filters. Filters were transferred onto Petri plates containing absorbent pads to which 2 ml of m-FC media (Millipore) was added. Plates were incubated at 37°C for 24h, photographed and counted. Colonies with a blue coloration were identified as fecal coliform bacteria. Additional 1 L samples (n=3 per site) of water were collected for PAH analysis. These samples were filtered through pre-conditioned Waters Oasis HLB cartridges (20 cc/1 g) until dry. Cartridges were kept cold prior to and during transport to the University of Mississippi, where they were rinsed with 10 ml ddH_2O, then eluted with methylene chloride (16 ml) into amber vials. Extracts were evaporated to dryness, weighed, and solubilized in 200 µl isooctane. Samples were run on an Agilent gas chromatograph coupled to a mass spectrometer, using the method described by Wade *et al.* (1993). PAHs within our samples were quantified against a standard curve comprising 16 standards in solution (UltraScientific, Cat. No. US-106N-4) from 0.05 to 0.5 ppm.

Data analysis

Species abundance data were arranged in a species by quadrat matrix and analyzed using the PRIMER software package (Plymouth Routines in Multivariate Ecological Research, version 5.1.2; Clarke and Warwick 1994). A similarity matrix was calculated using the fourth-root of the Bray-Curtis similarity index. Analyses of similarity (ANOSIM) were used to compare similarity within and among sites. Non-metric multi-dimensional scaling was used to generate a two-dimensional ordination of the communities. For each quadrat, the number of species, the number of individual sponges, the Shannon index of diversity (H'), and Pielou's measure of evenness (J') were calculated (Magurran 1988). These four indices were compared among the three sites using Kruskal-Wallis tests. Post-hoc means comparisons were performed using Mann-Whitney U tests. Rarefaction (species accumulation) curves were generated using the EstimateS software package (Colwell 2004). Nutrient concentrations at the three sites on each date were compared using Kruskal-Wallis tests. The effects of date and site on concentrations of nitrate and phosphate were analyzed using two-way non-parametric ANOVAs on rank-transformed data. Counts of fecal coliform bacteria and concentrations of individual PAHs among the three sites were compared using Kruskal-Wallis tests.

Results

Surveys

Overall, 51 species (Table 1) and 2532 individual sponges were identified in the 45 quadrats. Analysis of similarity (ANOSIM) indicated significant differences in sponge community structure among the three sites (Fig. 2; ANOSIM, R=0.705, P<0.001). Punta Caracol and Casa Blanca appeared to be more similar to each other than to Saigon, however, pair-wise comparisons indicated significant differences among all three sites (P<0.001 for each pair). Most species (41.2%) were observed at all three sites; however, 33.3% of species were observed from only a single site. Of the species found at two sites (25.5%), a greater number (6) were seen at Punta Caracol and Casa Blanca than were seen at either of those sites and Saigon (4 and 3 for Punta Caracol and Casa Blanca, respectively).

Saigon had significantly fewer species per quadrat than Punta Caracol, which had significantly fewer species per quadrat than Casa Blanca. The total number of individuals per site and the number of individuals per quadrat were similar between Saigon and the more distant upstream site, Punta Caracol (Table 2), and were significantly lower than at Casa Blanca. Evenness (J) was significantly reduced at Saigon, compared to the other two sites (Table 2), and diversity (H') differed significantly at all three sites, with Saigon having the lowest diversity and Casa Blanca the highest. Rarefaction (species-area) curves also indicate that the total number of species at Saigon is lower than at the other two sites (Fig. 3).

Dominant species also differed among sites, with one of the five most abundant species at Saigon (*Hymeniacidon* sp.) considered rare at the upstream sites (Fig. 4). Only *Niphates erecta* was among the five most dominant species at all three sites. *Aplysina fulva*, which dominated the reefs at Casa Blanca and Punta Caracol, was rare at Saigon. *Chondrilla nucula* and *Neopetrosia subtriangularis*, two of the dominant species at Casa Blanca, were rare or completely absent at the other two sites

Lesions on sponges were observed at all three sites. ARBS was present at all three sites, but prevalence was higher and more variable at Saigon (Fig. 5). However, the quadrat data indicate that the number of *Aplysina* rope sponges at Saigon (n=8) was negligible compared to the other two sites, where they were among the dominant species (n=127 and 272 at Punta Caracol and Casa Blanca, respectively). Other types of lesions were observed on *Iotrochota birotulata* (Punta Caracol), *Plakortis angulospiculatus* (Punta Caracol and Casa Blanca), *Amphimedon compressa* (Casa Blanca) and *Neofibularia nolitangere* (Saigon).

Environmental variables

Comparisons of nutrient concentrations among sites at each date are shown in Table 3. Nitrate concentrations varied greatly between the two sampling periods, but did not vary

Table 1: Distribution and abundance of sponge species identified in the quadrats from three sites in Bocas del Toro, Panama. Numbers indicate number of individual sponges (or ramets) in 15 1-m^2 quadrats at each site.

Species	Punta Caracol	Casa Blanca	Saigon
Aiolochroia crassa (Hyatt, 1875)	23	11	1
Amphimedon compressa Duchassaing and Michelotti, 1864	34	36	11
Aplysina cauliformis (Carter, 1882)	33	92	0
Aplysina fulva (Pallas, 1766)	94	180	8
Aplysina lacunosa (Pallas, 1766)	8	7	2
Chondrilla cf. *nucula* Schmidt, 1862	9	265	0
Cinachyrella alloclada (Uliczka, 1929)	9	0	4
Cliona aprica Pang, 1973	21	35	12
Cliona delitrix Pang, 1973	3	5	3
Dragmacidon reticulatum (Ridley and Dendy, 1886)	6	7	5
Ectyoplasia ferox (Duchassaing and Michelotti, 1864)	0	22	0
Haliclona (Halichoclona) vansoesti de Weerdt, de Kluijver and Gomez, 1999	9	0	0
Haliclona (Rhizoniera) curacaoensis (van Soest, 1980)	0	4	2
Haliclona sp.	0	90	0
Haliclona sp. (unidentified)	1	0	0
Halisarca caerulea Vacelet and Donadey, 1987	3	42	1
Hymeniacidon sp. (unidentified)	16	8	303
Hyrtios proteus Duchassaing and Michelotti, 1864	0	3	0
Iotrochota birotulata (Higgin, 1876)	10	1	6
Ircinia campana (Lamarck, 1814)	1	1	0
Ircinia felix (Duchassaing and Michelotti, 1864)	8	10	8
Ircinia strobilina (Lamarck, 1816)	1	1	3
Ircinia sp. (undescribed)	1	0	0
Lissodendoryx (Lissodendoryx) colombiensis Zea and van Soest, 1986	2	3	0
Monanchora arbuscula (Duchassaing and Michelotti, 1864)	21	30	4
Mycale (Arenochalina) laxissima (Duchassaing and Michelotti, 1864)	6	0	0
Mycale (Carmia) microsigmatosa Arndt, 1927	1	0	4
Mycale (Mycale) laevis (Carter, 1882)	26	68	88
Neofibularia nolitangere (Duchassaing and Michelotti, 1864)	6	0	0
Neopetrosia carbonaria (Lamarck, 1814)	0	15	1
Neopetrosia subtriangularis (Duchassaing, 1850)	0	98	0
Niphates caycedoi (Zea and van Soest, 1986)	4	0	16
Niphates erecta Duchassaing and Michelotti, 1864	52	98	28
Oceanapia nodosa (George and Wilson, 1919)	1	5	3
Petrosia (Petrosia) pellasarca (de Laubenfels, 1934)	3	2	2
Placospongia intermedia Sollas, 1888	1	8	0
Plakortis angulospiculatus (Carter, 1882)	10	13	9
Plakortis halichondrioides (Wilson, 1902)	0	6	0
Spirastrella hartmani Boury-Esnault *et al.* 2000	0	40	0
Spirastrella sp. (unidentified)	75	61	22
Spongia (*Spongia*) *pertusa Hyatt, 1877*	1	0	*1*
Svenzea zeai (Alvarez, van Soest and Rützler, 1998)	0	0	1
Tedania (Tedania) ignis (Duchassaing and Michelotti, 1864)	4	9	0
Verongula reiswigi Alcolado, 1984	0	1	0
Verongula rigida (Esper, 1794)	11	26	2
Xestospongia sp.	86	6	4
Xestospongia proxima (Duchassaing and Michelotti, 1864)	0	8	0
Xestospongia rosariensis Zea and Rützler, 1983	0	11	18
Unidentified sp. 1	0	0	1
Unidentified sp. 2	0	1	0
Unidentified sp. 3	0	12	0

significantly among sites (Table 3; Two way non-parametric ANOVA, df = 1, F = 66.441, $P < 0.0001$ for date, df = 2, F = 0.099, P = 0.9067 for site). Phosphate concentrations did not vary significantly over time but there was a trend towards Punta Caracol having higher concentrations than the other two sites (Two way non-parametric ANOVA, df = 1, F = 2.059, P = 0.1768 for date, df = 2, F = 3.629, P = 0.0585 for site). When analyzed separately, phosphate concentrations varied significantly among sites in November, but not in July (Table 3). Nitrite concentrations also varied significantly among sites in November (Table 3). For both phosphate and nitrite concentrations in November, lower concentrations were found at Casa Blanca that at Punta Caracol and Saigon, which were similar to each other. Fecal coliform counts were highly variable within and among sites; however, a statistically significant difference between coliform numbers was not detected (Table 3). Ten PAHs were detectable in our samples at low concentrations that were highly variable even among

Fig. 2: Non-metric multi-dimensional scaling ordination of sponge diversity in 15 1-m^2 quadrats at each of the three sites in Bocas del Toro, Panama; stress = 0.2. Each symbol represents the community composition based upon a single quadrat. Closer symbols indicate more similar assemblages.

replicates within sites (Table 3). Only for phenanthrene and anthracene was there a trend towards differences among sites (Kruskal-Wallis, df = 2, H = 5.600, P = 0.0608 for both), with Punta Caracol having significantly higher concentrations of both compounds than Casa Blanca, and Saigon being intermediate (Table 3).

Discussion

Site

The reefs of Bahía Almirante in Bocas del Toro, Panama, are well-developed and host high biodiversity (Collin *et al.* 2005). However, the region receives a significant amount of rainfall, usually in severe episodic downpours, resulting in excessive runoff of terrestrial sediment and potentially agricultural and other forms of land-based pollution. These inputs, combined with low flushing from the Caribbean Sea (Carruthers *et al.* 2005), result in prolonged residence times and exposures of resident organisms to sediments, nutrients and potential pollutants. Most of the towns and villages in the region are small, but they are built directly beside the water, with little or no treatment for sewage or other inputs.

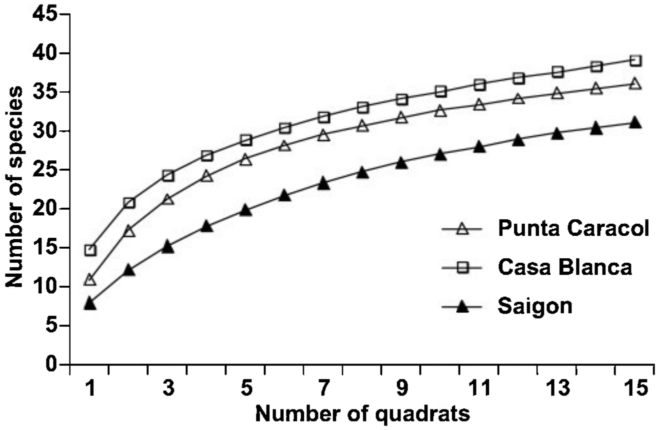

Fig. 3: Rarefaction (species-area) curves based on species counts in 1-m^2 quadrats at the three sites in Bocas del Toro, Panama.

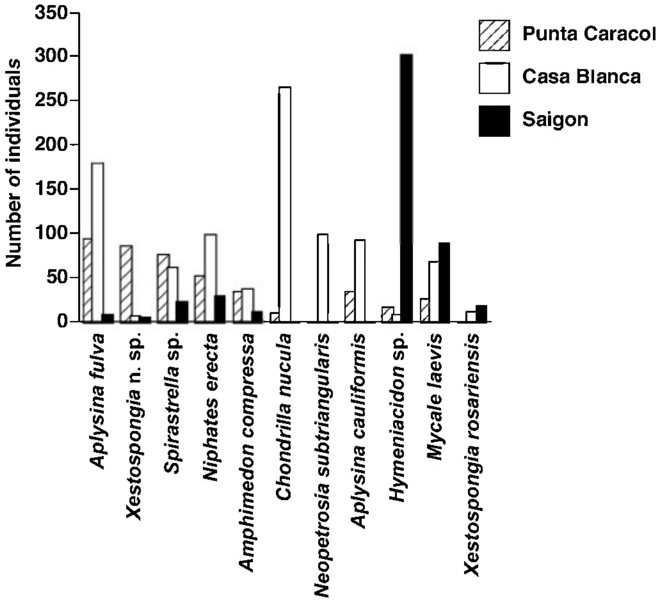

Fig. 4: Abundance of the five most dominant sponge species at the three sites in Bocas del Toro, Panama. Values are total numbers of individuals in 15 1-m^2 quadrats at each site.

Table 2: Comparison of sponge diversity, abundance and community indices from 15 1-m^2 quadrats at each of the three sites in Bocas del Toro, Panama. Values are means with standard errors in parentheses. Degrees of freedom (df), H and P values are from Kruskal-Wallis tests. Superscripts indicate groups that differ significantly from each other, based on post-hoc pairwise Mann-Whitney U tests at $P < 0.05$.

	Punta Caracol	Casa Blanca	Saigon	df	H	P
Number of species/quadrat	10.8 (3.5)^a	14.6 (4.1)^b	7.8 (2.6)^c	2	17.937	0.0001
Total species	36	39	31			
Number of individuals/quadrat	40 (23.8)^a	90.7 (39.5)^b	38.1 (19.2)^a	2	17.267	0.0002
Total individuals	600	1360	572			
J (evenness)	0.85 (0.05)^a	0.84 (0.04)^a	0.68 (0.15)^b	2	15.723	0.0004
H' (diversity)	1.99 (0.27)^a	2.21 (0.31)^b	1.39 (0.44)^c	2	24.230	< 0.0001

Table 3: Comparison of nutrient concentrations (in micromolar = μM), counts of fecal coliform colonies on culture plates, and PAH concentrations (in parts per million = ppm) from replicate water samples (n = 3) at the three sites in Bocas del Toro, Panama. Values are means with standard errors in parentheses. n.d. = not detectable. Degrees of freedom (df), H and P values are from Kruskal-Wallis tests. Superscripts indicate groups that differ significantly from each other, based on post-hoc pairwise Mann-Whitney U tests at P < 0.05.

	Punta Caracol	Casa Blanca	Saigon	df	H	P
Nutrients (μM)						
Nitrate (November)	0.40 (0.013)	0.43 (0)	0.41 (0.017)	2	1.787	.4093
Nitrite (November)	0.25 (0.012)^{a}	0.16 (0.012)^{b}	0.25 (0.009)^{a}	2	5.744	.0506
Phosphate (November)	0.61 (0.017)^{a}	0.52 (0.015)^{b}	0.56 (0.01)^{ab}	2	6.330	.0422
Nitrate (July)	0.22 (0.053)	0.16 (0)	0.22 (0.053)	2	1.143	.5647
Ammonia (July)	n.d.	n.d.	0.057 (0.057)	2	2.000	.3379
Phosphate (July)	0.81 (0.21)	1.33 (1.33)	0.14 (0.14)	2	3.840	.1566
Fecal Coliform Counts	12.67 (7.22)	1.75 (1.75)	8.0 (4.16)	2	2.617	.2702
PAHs (ppm)						
phenanthrene	0.025 (0)^{a}	n.d.^{b}	0.008 (0.008)^{ab}	2	5.600	.0608
anthracene	0.035 (0)^{a}	n.d.^{b}	0.012 (0.012)^{ab}	2	5.600	.0608
benz[a]anthracene	0.018 (0.018)	0.023 (0.023)	0.018 (0.018)	2	0.095	.9535
chrysene	0.015 (0.015)	0.02 (0.02)	0.015 (0.015)	2	0.095	.9535
benzo[b]fluoranthene	n.d.	0.018 (0.018)	n.d.	2	2.000	.3679
benzo[k]fluoranthene	n.d.	0.023 (0.023)	n.d.	2	2.000	.3679
benzo[a]pyrene	n.d.	0.027 (0.027)	n.d.	2	2.000	.3679
indeno[1,2,3-cd]pyrene	0.02 (0.02)	0.027 (0.027)	n.d.	2	1.167	.5580
dibenzo[a,h]anthracene	n.d.	0.003 (0.003)	0.02 (0.02)	2	1.167	.5580
benzo[ghi]perylene	n.d.	0.005 (0.005)	0.01 (0.01)	2	1.167	.5580

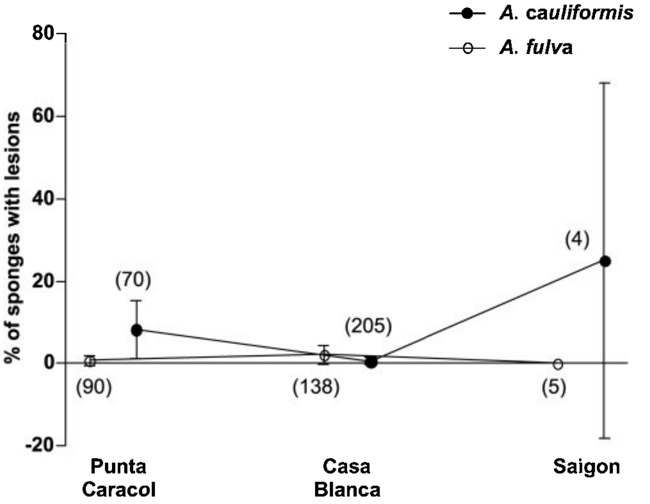

Fig. 5: Prevalence (percent of sponges with lesions) of *Aplysina* Red Band Syndrome on *Aplysina cauliformis* and *A. fulva* at each of the three sites in Bocas del Toro, Panama. Points represent mean (± standard error) prevalence along three 10 x 1 m transects. Total number of *Aplysina* spp. sponges surveyed along the transects is shown in parentheses.

Carruthers *et al.* (2005) examined lagoonal scale processes in Bocas del Toro, and although Bahía Almirante had less terriginous input than nearby Laguna de Chiriqui, the entire region was found to have high levels of nutrients and sediment. Aronson *et al.* (2004) reported a phase shift from *Porites*-dominated to *Agaricia*-dominated reefs in Bahía Almirante since the 1970s, and attributed this primarily to changes in water quality. Thus, the coral reef communities found in this region at present are probably adapted to a combination of these natural and anthropogenic environmental stressors.

Surveys

The sponge fauna on the reefs and mangrove habitats in Bocas del Toro, Panama is estimated at approximately 120 species (Diaz 2005). We found 51 species of sponges in our quadrats at three coral reef sites within this region. In general, the species found in Bocas del Toro and in our quadrats are widely distributed throughout the Caribbean (Diaz 2005). As seen in other studies (Diaz 2005), while many species were found at all three sites, one third of all species were observed at only a single site. *Niphates erecta*, for example, was one of the five most dominant species at all of our sites, and Diaz (2005) also found this species to be common at all 14 sites surveyed in both reef and mangrove habitats in Bocas del Toro. By contrast, *Hymeniacidon* sp. was dominant at Saigon but virtually absent from our quadrats at the other two sites, whereas *Chondrilla nucula* and *Neopetrosia subtriangularis* were only dominant at Casa Blanca.

Saigon had lower numbers of sponge species per quadrat and overall, as well as lower community evenness and diversity compared to Punta Caracol and Casa Blanca. There is a widely-observed trend towards species-rich, highly diverse communities at environmentally healthy sites (Birkeland 1997, Bertness *et al.* 2001), suggesting that Saigon may be exposed to a higher degree of stress than the other two sites. Organic and inorganic pollution have been demonstrated to reduce sponge species diversity in Cuba (Alcolado and Herrera 1987), Brazil (Muricy *et al.* 1991, Monteiro and Muricy 2004) and France (Muricy 1991). By contrast, organic pollution increased cover and abundance of sponges in Colombia (Zea 1994), possibly because sponges are more tolerant of high turbidity than other reef macro-organisms. Muricy (1989) found reduced numbers of sponge species, species abundance and dominance, percent cover, density of individuals and species diversity indices at an organically polluted bay in Brazil, as compared to two nearby clean sites, and Alcolado (1994) found that organically polluted sites near Havana, Cuba, had lower heterogeneity and diversity than unpolluted sites. Similar trends were obtained at the site of a nuclear power plant discharge in Brazil (Vilanova *et al.* 2004), where the main stressors included thermal pollution, chlorine and high water flow. In the present study, certain community characters, such as overall number of sponges and number of sponges per quadrat, were similar between Saigon and Punta Caracol. These data suggest either that community indices alone cannot be used to indicate ecological stress or that environmental conditions at Saigon and Punta Caracol are more similar to each other than at Casa Blanca, even though the latter site is located in between the other two.

In addition to changes in diversity and abundance, sponge community composition and species dominance often differ in response to environmental stress. In the present study, species composition was more similar among the two upstream sites than between those two sites and Saigon, further suggesting that Saigon is exposed to different environmental factors than the other two sites. In addition, species that were dominant at Saigon were rare or absent from the other two sites and vice versa. Among these, *Chondrilla nucula*, is known to be sensitive to stress (Alcolado and Herrera 1987, Muricy 1989, Vilanova *et al.* 2004), and was completely absent from the reef at Saigon. However, *C. nucula* was also not common at Punta Caracol and could easily have been missed in surveys. The absence of this species may not be sufficient to indicate a stressed site, but its presence likely indicates cleaner water. *Neopetrosia subtriangularis* was only found at Casa Blanca and may also be indicative of more favorable water conditions. *Aplysina cauliformis* and *A. fulva* were common at both upstream sites and absent or rare at Saigon. Preliminary experiments have indicated that *A. cauliformis* is highly sensitive to nutrient enrichment (D. Gochfeld, unpubl. data: Bahamas 2006), suggesting at least one possible stressor that could account for the differences among sites. In contrast, *Hymeniacidon* sp. occurred predominantly at Saigon. Previous studies have demonstrated that the degree of dominance by particular sponge species can also differ among sites, with stressed sites typically dominated by fewer species (Alcolado and Herrera 1987, Muricy 1991, Alcolado 1994, Monteiro and Muricy 2004). At Saigon, the sponge community was dominated by *Hymeniacidon* sp., with *Mycale laevis* a distant second and other species dropping off numerically thereafter. By contrast, although *Chondrilla nucula* was a clear dominant species at Casa Blanca, four other species were each represented by > 90 individuals at that site. The sponge community at Punta Caracol was much more even, with the five most dominant species each represented by fewer than 100 individuals.

Syndromes or diseases of sponges were present at all three sites. In addition to ARBS, other types of lesions were observed on *Iotrochota birotulata*, *Amphimedon compressa*, *Plakortis angulospiculatus* and on one large colony of *Neofibularia nolitangere*. It is not possible to identify the causes of these lesions in the absence of any active signs of disease without detailed microbiological and microscopic study. Aside from these observations, ARBS was the main syndrome observed. ARBS was present at all three sites, but disease prevalence was highest at Saigon, which also had the lowest sponge diversity. However, this result is confounded by the fact that the affected sponges, *Aplysina cauliformis* and *A. fulva* were very rare on the reef at Saigon, indicating the need for further study on the dynamics of this sponge disease.

Environmental parameters

All three sites may be affected by specific stressors that have not yet been identified. We hypothesized that nutrients and fecal coliform bacteria from sewage, and PAHs from road runoff could be likely candidate pollutants at these sites, but these have not yet been fully characterized. Sewage effluent can result in turbidity, eutrophication, pathogenic microbes and pharmaceuticals in the environment, and is known to cause changes in the structure and distribution of many biological communities, including those dominated by sponges (Rose and Risk 1985, Muricy 1989, Ward-Paige *et al.* 2005). In an effort to characterize the mechanisms by which sewage effluent affects these organisms, Roberts *et al.* (2006) found declines in growth, reproduction and chlorophyll *a* concentrations in the phototrophic sponge *Cymbastela concentrica* when exposed to shade, silt and salinity gradients, but not to nutrients alone. Muricy (1989) found a significant correlation between several pollution parameters (water transparency, oil and coliform levels) and community indices, identifying *Mycale microsigmatosa* as a species that is tolerant of variable conditions, *Ulosa ruetzleri* and *Amphimedon viridis* as low-sensitivity species, and *Aplysina fistularis, Tedania ignis, Chondrilla nucula* and *Polymastia* sp. as highly sensitive species due to their total or near absence at highly polluted sites. Certain species, such as *Scopalina ruetzleri* exhibited an increase in the proximity of runoff in Colombia (Zea 1994), but were less abundant at polluted sites in other studies (Alcolado and Herrera 1987, Muricy 1989), a situation that may reflect the variable physiology of these sponges or differences in overall levels or types of stress (Muricy *et al.* 1991, Zea 1994).

Sponges are known to exhibit specific responses to other types of pollution. For example, *Spongia officinalis* can metabolize certain polychlorobiphenyl contaminants (Perez *et al.* 2003). Sponges can also concentrate metals from their environment (Cebrian *et al.* 2003, Perez *et al.* 2004), and have been found to produce metallothionein-like proteins that may serve as useful biomarkers (Berthet *et al.* 2005). To date, no studies have elucidated specific sponge responses to PAHs, but PAHs are known to induce phototoxicity (Peachey and Crosby 1995) and reduced reproduction (Guzman and Holst 1993) in corals.

Since we observed significant differences in sponge community structure among sites that appear to have similar, and relatively low, abundances of corals and algae, we believe that sponges may serve as sensitive bioindicators of natural and anthropogenic impacts on these reef communities. Based upon their restricted distributions, several sponge species are promising candidates as bioindicators in the Bocas del Toro region. Our measurements of potential pollutants represent a snapshot in time at sites that exhibit a high degree of temporal variability in nutrients and other water quality parameters (P. Gondola, pers. comm.). The combination of elevated nutrient levels, fecal coliform bacteria and several PAH compounds, indicates that pollution from storm-water runoff is a concern at all three of the sites studied. Clearly, a further analysis of natural and anthropogenic stressors would aid in identifying the specific causes of the differences in sponge community structure and disease prevalence observed among these sites. Nonetheless, in this system, sponges appear to be more sensitive bioindicators than other components of these coral reefs.

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